

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
LEUNG *et al.*

Serial No.: 09/988,013

Filed: November 16, 2001

Title: IMMUNOCONJUGATES AND HUMANIZED
ANTIBODIES SPECIFIC FOR B-CELL
LYMPHOMA AND LEUKEMIA CELLS

VIA EFS-WEB

Group Art Unit: 1643

Examiner: David Blanchard

Attorney Docket No.: IMMU:014US2

Confirmation No.: 7681

DECLARATION

COMMISSIONER FOR PATENTS
P. O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, Bryan Wilson, being duly warned, declare as follows:

I am a partner with Morrison and Foerster LLP. We represent Immunomedics, Inc. in connection with litigation in which former employee Dr. Shui-On (“Shawn”) Leung is the plaintiff and cross-defendant (*SinoMab Bioscience Ltd., et. al. v. Immunomedics, Inc.*, Delaware Chancery Court Civil Action No. 2471-VCS).

On October 5, 2009, we forwarded to Gregory Lantier of Wilmer Hale a copy of a Rule 131 declaration, which is attached, to be used in responding to the citation of Harris (WO 94/09136) in this case. Mr. Lantier and his firm represent Dr. Leung in connection with the above-mentioned litigation.

Mr. Lantier informed us that he presented the Rule 131 declaration to Dr. Leung for execution.

On October 13, 2009, Mr. Lantier responded to us that Dr. Leung had suggested proposed changes to the proffered Rule 131 declaration.

On October 21, 2009, a revised Rule 131 declaration, which also is attached, was forwarded to Mr. Lantier.

Mr. Lantier told us that he presented the revised Rule 131 declaration to Dr. Leung for execution. On November 5, 2009, Mr. Lantier advised us that Dr. Leung again had refused to sign the proffered Rule 131 declaration.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12/16/2009
Date

Bryan Wilson
Bryan Wilson

First Rule 131 Declaration

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VIA EFS-WEB

DECLARATION UNDER 37 CFR § 1.131

COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450

Sir:

I, Shui-on Leung, being duly warned, declare as follows:

I am a co-inventor of the above-identified application.

Attached is a copy of a monthly progress report submitted by me while I was employed by Immunomedics, Inc. as a research scientist, reporting work done at Immunomedics. The date on the monthly progress report has been redacted, but was before April 28, 1994.

The report discusses chimeric LL2, humanized LL2.1 (HuLL2) and humanized LL2.2 (mutHuLL2). The humanized LL2 antibodies were made by selecting framework regions from a first human antibody for the light chain and selecting framework regions from second and third human antibodies for the heavy chain based on a sequence comparison. The heavy chain FR1, FR2 and FR3 were selected from the second human antibody and FR4 was selected from the third human antibody. The heavy chain FR4 for the humanized antibody was selected from the human NEWM antibody, the light chain framework regions were selected from the human REI antibody, and the heavy chain FR1, FR2 and FR3 were selected from the human EU antibody.

Also attached is a copy of a page from my Immunomedics lab notebook entitled "DNA Sequence of LL2 Gamma Chain Variable Region as Cloned Out Using CHIA and VH1Back Primers in a PCR Reaction," recording work done at Immunomedics. The date on the laboratory notebook page has been redacted, but was before April 28, 1994.

The page from the laboratory notebook shows CDR-grafting of the LL2 heavy chain and includes notations at the bottom that EU is selected for FR1-3. Looking at the left of the start of the murine LL2 sequence, there are hand written residues. Those which differ for NEWM are hand written above, and those that differ for KOL are written below, the murine LL2 amino acid sequence. Those positions where the EU sequence amino acids differ from the murine LL2 sequence are written between the nucleotide sequences. In FR4 the first three critical amino acid residues of murine LL2 are WGQ. The amino acid residues of EU are EYN, and therefore EU was not a good choice for FR4. Both NEWM and KOL were a better fit for FR4 than EU because they have fewer residues that differ and because the first three critical residues are the same as those in the murine framework.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date

Shui-on Leung, PhD.

Humanization Program

Progress Report

Name:

Dr. Shawn Leung

Supervisor:

Dr. Hans Hansen

MN14 (Dr. Leung)

Confirmation of the Heavy Chain Sequence- in the construction of the heavy chain sequence, several rounds of PCR reactions were carried out. To ensure no undesirable mutation was accidentally introduced, three clones containing the heavy chain PCR products (heavy chain staging vector) were sequenced. One of which indicated deleterious mutation and the other two appeared to be correct. The clones with the correct sequence were used for constructing the MN14 Fab bacterial expression vector.

Confirmation of the Light Chain Sequence- similarly, the construction of the light chain sequence entailed several rounds of PCR reaction. Intensive sequencing reactions were carried out on three positive clones containing the light chain PCR products (light chain staging vector). All appeared to be of the correct sequence of my original design. Medium scale DNA was prepared for use as insert supplies.

Construction of the Final Bacterial Expression Vector for Humanized MN14 F(ab)'₂- the staging vector containing the heavy chain PCR product was digested with BglII and XbaI. The DNA was purified and served as the cloning vector. Light chain sequence was excised from the light chain staging vector by digestion with BglII and XbaI. The 750 bp insert was then gel-purified and was allowed to ligate with the cloning vector. Transformants were subjected to mini-DNA preparation analysis. Clones releasing inserts of 1.5 kb after HindIII/XbaI digestion were identified as positives.

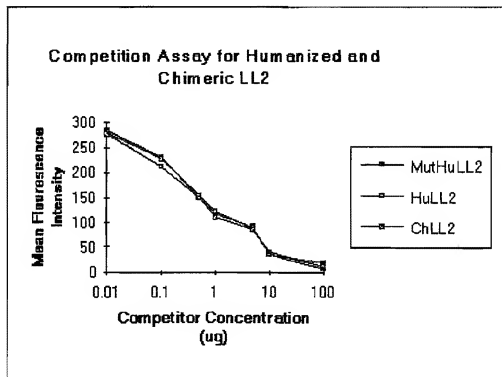
Confirmation of the Humanized MN14 F(ab)'₂ Bacterial Expression Vector Sequence- since the genes encoding the humanized MN14 F(ab)'₂ sequence spanned about 1.5 kb, we had to design 10 addition sequencing primers if the full sequence was to be elucidated. These primers were obtained from Genosys. Intensive sequencing reaction has shown that the construct contains sequence as my original design. The construct is named as MN14pBEV. It contains a LacZ promoter (IPTG

inducible) and a dicistronic gene containing both the heavy and light chain sequence. Pel B sequence is used as the signal peptide to facilitate transport into the periplasm. Plasmid DNA was prepared for expression studies.

With the availability of the bacterial expression vector, we are in a position to plan experiments to optimize the conditions for its expression.

LL2 (Dr. Leung)

Competition Studies on Humanized LL2- as described in the February report, it has been shown that both murine and chimeric antibodies exhibit similar binding affinity. These were further confirmed by Dr. Shevitz when radioimmunoassay was carried out on murine and chimeric LL2. Thus, chimeric LL2 is used as the standard for competitive binding studies on humanized LL2. Two versions of humanized LL2 were purified as previously described. 1 ug of chimeric LL2, humanized LL2.1 (HuLL2) and humanized LL2.2 (MutHuLL2), respectively, were competed with different concentrations of murine LL2. The level of the chimeric or humanized antibodies binding to the Raji cells post-competition was then detected by FITC-conjugated goat anti-human Fc antibodies in a FACscan analysis. It should be noted that the decrease of fluorescence intensity is inversely proportional to the affinity of the antibody tested. As shown in the following fig., there is basically no difference in affinity between the chimeric and humanized antibodies. The experiment was repeated three times.



In conclusion, the humanized LL2, both version 1 and 2, are similar, if not identical, to the chimeric and murine LL2 in terms of binding affinity.

Purification of Chimeric LL2 with the VK Glycosylation Site Removed- clones obtained from transfection #45 and 46 are in general low producers. Clone 45.2H8, which secretes a chimeric LL2 with no glycosylation in the light chain, was upscaled for protein A purification. The purified antibody, when subjected to SDS-PAGE analysis, showed no retarded migration nor multiple bands in the light chain. Rather, it ran as a discrete band running at a position characteristic of an unglycosylated light chain. This proves our design to eliminate the glycosylation site in the FR1 sequence of VK chain to be successful. FACscan analysis will be performed to evaluate the effect of the loss of glycosylation on the binding affinity of the antibody.

Mix and Match Transfection Experiment- positives identified for transfection #40, 41 and 42 were subjected to ELISA assay to determine the level of secretion when cells are confluent.

Positive clones	Construct Combination	Ab Conc. in Supernatants
40.1E4	HuLLKh/LLGg	0.05 ug/ml
40.2B11		0.3 ug/ml
41.1D2	LLKh/MutHuLLGg	0.5 ug/ml
41.2B3		0.2 ug/ml
41.2C3		0.2 ug/ml
41.2D3		0.08 ug/ml
42.1H8	LLKh/HuLLGg	0.2 ug/ml
42.1E11		0.05 ug/ml

Clone 40.2B11, 41.1D2 and 42.1H8 are the highest producers amongst other positives. They were selected for upscaling and will be purified by Protein A column. Once the purified antibodies are available, a competition assay will be performed to determine the effect of individual light or heavy chain on the binding affinity of LL2 after humanization.

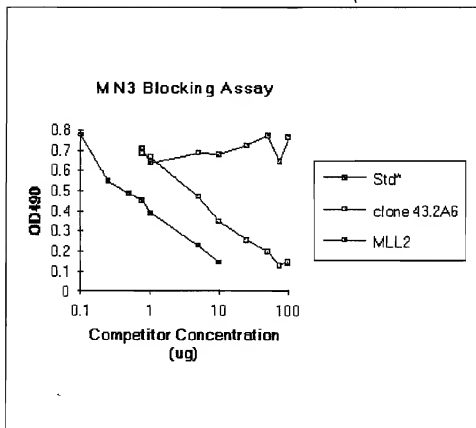
MN3(Dr. Leung)

Humanization of MN3 Light Chain- as noted previously, one of the oligos needed for the humanization, oligo I, was defective. Another oligo, named as oligo I', was re-designed and synthesized. After purification, the oligo I' was subjected to PCR

reaction with oligos 19 & 20 as primers. Good PCR product of I' (150 bp) was obtained. The PCR product of I' was digested with BspEI & BglII and gel-purified. Similarly, the PCR product of J was digested with BspEI & PvuII and gel-purified. They were then ligated to the staging vector, VKpBR(PvuII/BclII), in a three fragment ligation. Clones with the right insert size of 300 bp were obtained. Three of which were sequenced intensively. Sequence of the humanized MN3 light chain was confirmed to be identical to my original design. The VK fragment was then excised from the staging vector and subcloned into the final expression vector, forming the humanized MN3 light chain expression vector, HuMN3Kh. To confirm the identity of the final construct, sequencing reactions were performed. Plasmid preparation of HuMN3Kh was prepared and ready for use in transfection.

MN3 Chimeric Light and Humanized Heavy Chain Transfection Studies- in transfection #43 and 44, only #43 gives out clones that exhibited some blocking activities. Clone 43.2A6 was upscaled and antibody purified by Protein Column. The final concentration of the purified antibody was determined by ELISA assay. MN3 blocking assay was performed, with murine ~~LL2~~ as the standard (Std) and murine LL2 as the negative control. The result is summarized below.

MN3.



The experiment was repeated three times and the result indicated that the humanized MN3 heavy chain exhibits decreased blocking activities, approximately by 10 folds. In order to restore the binding affinity of the humanized MN3 heavy chain, more framework mutations have to be introduced.

/sl

CC. Dr. David Goldenberg
Mr. David Ortlieb
Dr. Carl Pinsky
Dr. Ken Chang
Mr. Gil Rudman

(Linear) MAP of: Ll2-G check: 8584 from: 1 to: 348
DNA SEQUENCE OF LL2 GAMMA CHAIN VARIABLE REGION AS CLONED OUT
USING CHIA AND VH1BACK PRIMERS IN A PCR REACTION.

13(11:6) Gk k

1 TAGGTC CAGCTCAGGAGT CAGGGGTGAACTGTC AAAA CCTGGG CCTCAGTGAAGATG 60
ATCCAGGT CAGCTCCTCAGTCCCGACTTGACAGTTTGGACCCCGAGTCACTTCTAC
* V Q E Q E S G A E L S K P G A S V K M
TCTGCAAGGCTCTGGGTACACCTTTACTAGCTACTGGCTGCACGTGATAAAGACAGG
61 AGGACGTTCCGAAGACCGATGTGGAATGATCGATGACCGACGTGACCTATTGTCTCC 120
S C K A S G Y T F T S Y W L H W I K R P
CCTGGACAGGCTCTGGAATGGATTGGATACATTAATCCTAGGAATGATTATACGTAGTAC
121 GGACCTGTC CAGACCTTACCTAACCTATGTAATTAGGATCCTTACTAATATGACTCATG 180
P G Q G L E W I G Y I N P R N D Y T E Y
AATCAGAACTTCAAGGACAAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTAC
181 TTAGTCTTGAAGTTCCGTGTTCCGGTGTAAGTCTGTTTAGGAGGTCGTGTCGGGATG 240
N Q N F E D K A T L T A D K S S T A Y
ATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAAGGAT
241 TACGTTGACTCGTCGGACTGTAGACTCCTGAGACGTGAGATAATGACAGCTTCTCCCTA 300
M Q L S S L T S E D S A V Y Y C A R R D
ATTACTACGTTCTACTGGGGCCCAAGGCACCACTCTCACAGTCTCCTCG
301 TAATGATGCAAGATGACCCCGGTTCCGTGTTGAGAGTGTGACAGGAGC 348
I T T F Y W G Q G T T L T V S S

This sequence has been checked to be identical to :
Miniprep # 1, 6, 10. [redacted] - primers T7 and MHGconst to give full length sequence from both directions.
Miniprep # L4 ([redacted]) - primers T7 and MHGconst to give partial sequence information.
Miniprep # L1, L2, L3 and L12 [redacted] -primers Reverse primer and VH1-For to give partial sequence information.
Miniprep # 3 [redacted] - primers T7 and VH1-for to give sequence information up to 318 bp.

FR1 :	Eu: 8	FR2: Eu: 4	FR3: Eu: 12
	NEW: 15	NEW: 4	NEW: 17
	KOH: 15	KOH: 6	KOH: 16
	L	L	L

It matches much better with EU FRAMEWORK.

Second Rule 131 Declaration

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The report discusses experiments performed on chimeric LL2, humanized LL2.1 (HuLL2) and humanized LL2.2 (mutHuLL2). The chimeric and humanized LL2 antibodies of the progress report are those described in the present application.

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The page from the laboratory notebook shows sequence comparison of the LL2 heavy chain to three of the most commonly used human heavy chain frameworks for CDR grafting at the time. They were, namely, EU, NEW and KOL. A summary on the number of amino acid mismatches to the corresponding FR1, FR2 and FR3 of murine LL2 heavy chain was listed. It indicates at the bottom EU framework matches better with LL2. Looking at the left of the start of the murine LL2 sequence, there are hand written residues. Those which differ for NEWM are hand written above, and those that differ for KOL are written below, the murine LL2 amino acid sequence. Those positions where the EU sequence amino acids differ from the murine LL2 sequence are written between the nucleotide sequences. In FR4 the first three critical amino acid residues of murine LL2 are WGQ. The amino acid residues of EU at corresponding positions are EYN, and therefore, regardless of its best overall homology, choosing the whole EU framework for CDR-grafting was not a good choice. It was then rationalized that FR4 did not necessarily have to come from the same human heavy chain. Both NEWM and KOL were a better fit for FR4 than EU because they have fewer residues that differ and because the first three critical residues are the same as those in the murine framework.

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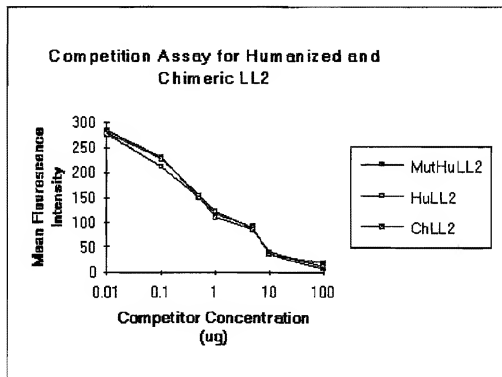
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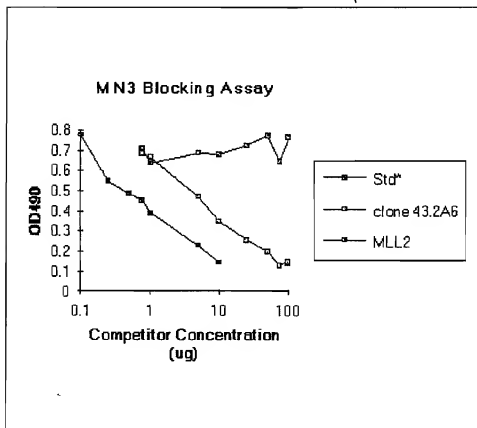
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(Linear) MAP of: Ll2-G check: 8584 from: 1 to: 348
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ATCCAGGTCGACGTCCTCAGTCCCGACTTGACAGTTTGGACCCCGAGTCACTTCTAC
* V Q E Q E S G A E L S K P G A S V K M
TCTGCAAGGCTCTGGGTACACCTTTACTAGCTACTGGCTGCACGTGATAAAGACAGG
61 AGGACGTTCCGAAGACCGATGTGGAATGATCGATGACCGACGTGACCTATTTGTCTCC 120
S C K A S G Y T F T S Y W L H W I K R P
CCTGGACAGGCTCTGGAATGGATTGGATACATTAATCCTAGGAATGATTATACGTAGTAC
121 GGACCTGTC CCGACAGCTTACCTAACCTATGTAATTAGGATCCTTACTAATATGACTCATG 180
P G Q G L E W I G Y I N P R N D Y T E Y
AATCAGAACTTCAAGGACAAGGCCACATTGACTGCAGACAAATCCTCCAGCACAGCCTAC
181 TTAGTCTTGAAGTTCCGTGTTCCGGTGTAACTGACGTCTGTTTAGGAGGTCGTGTCGGATG 240
N Q N F E D K A T L T A D K S S T A Y
ATGCAACTGAGCAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAAGAAGGAT
241 TACGTTGACTCGTCGGACTGTAGACTCCTGAGACGTGAGATAATGACAGCTTCTCCCTA 300
M Q L S S L T S E D S A V Y Y C A R R D
ATTACTACGTTCTACTGGGGCCCAAGGCACCACTCTCACAGTCTCCTCG
301 TAATGATGCAAGATGACCCCGGTTCCGTGCTGAGAGTGTCAGAGGAGC 348
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This sequence has been checked to be identical to :
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Miniprep # 3 [redacted] - primers T7 and VH1-for to give sequence information up to 318 bp.

FR1 :	Eu: 8	FR2: Eu: 4	FR3: Eu: 12
	NEW: 15	NEW: 4	NEW: 17
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	L	L	L

It matches much better with EU FRAMEWORK.